



ORIGINALES

Quantitative analysis of the interstitial mast cells in idiopathic mesangiocapillary glomerulonephritis type I

M. Danilewicz y M. Wągrowaska-Danilewicz

Department of Pathology (Morphometry Division) Medical University of Łódź, Poland.

SUMMARY

Fourteen renal biopsy specimens from patients with mesangiocapillary glomerulonephritis type I (MCGN-I) for whom both light and electron microscopy as well as immunofluorescence microscopy and full clinical data were available were examined quantitatively. As a control 10 biopsy specimens of kidneys removed because of trauma were used. Morphometric investigations were performed by means of a computer image analysis system to evaluate whether mast cells have a role in tubulointerstitial fibrosis in MCGN-I and to examine the relationship between mast cells and interstitial α -smooth muscle actin (α -SMA) expression as well as interstitial infiltrates.

The morphometric study revealed that the mean values of the interstitial tryptase positive cells, expression of α -SMA, interstitial volume, CD 68+, CD 45RB+, CD 43+ and CD 20+ cells were significantly increased in MCGN-I patients in comparison with control group. In MCGN-I group there were significant positive correlations between interstitial tryptase positive cells and interstitial expression of α -SMA, interstitial volume, serum creatinine as well as CD 43+ and CD 68+ cells. The correlations between interstitial tryptase positive cells and CD 45+, as well as CD 20+ cells did not reach statistical significance.

In conclusion, our findings demonstrate that mast cells are one of the constitutive cell types in the interstitium in MCGN-I. Additionally, significant positive correlations between interstitial mast cell count and relative interstitial volume as well as serum creatinine concentration suggest the role of these cells in the development of interstitial fibrosis which may contribute to the renal deterioration in patients with MCGN-I.

Key words: **Mast cells. Mesangiocapillary glomerulonephritis type I. Morphometry.**

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Correspondencia: Marian Danilewicz M.D., Ph.D.

ul. Zamenhofa 5 m. 4

90-431 Łódź, Poland

E-mail: hobo@psk2.am.lodz.pl

ANÁLISIS CUANTITATIVO DE LOS MASTOCITOS INTERSTICIALES EN LA GLOMERULONEFRITIS MESANGIOCAPILAR IDIOPÁTICA TIPO I

RESUMEN

Se examinaron 14 biopsias renales de enfermos con glomerulonefritis mesangiocapilar tipo I (GNMC-I) por microscopía óptica, electrónica e inmunofluorescencia, así como sus datos clínicos y analíticos. Como control se emplearon 10 muestras de nefrectomías por traumatismos renales. Se efectuaron investigaciones morfométricas mediante un sistema de imágenes computarizadas con el fin de valorar si los mastocitos juegan algún papel en la fibrosis intersticial de la GNMC-I y examinar la relación entre dichas células, los infiltrados intersticiales y la actina de músculo liso (α -SMA).

El estudio morfométrico reveló que los valores medios de las células intersticiales triptasa positivas, la expresión de α -SMA, el volumen intersticial, y las células CD 68+, CD 45RB+, CD 43+ y CD 20+ estaban significativamente aumentadas en comparación con el grupo control. En el grupo de GNMC-I existía una correlación significativa positiva entre las células intersticiales triptasa + y a la expresión intersticial de α -SMA, el volumen intersticial, la creatinina sérica y las células CD 43+ y CD 68+. Las correlaciones entre las células intersticiales triptasa positivas y las células CD 45+ y CD 20+ no alcanzaron significación estadística.

En conclusión, nuestros hallazgos demuestran que los mastocitos son uno de los tipos de células que forman el intersticio de las GNMC-I. Además, el hallazgo de unas correlaciones positivas significativas entre el recuento de células intersticiales y el volumen intersticial relativo y los niveles séricos de creatinina sugieren el papel de estas células en el desarrollo de fibrosis intersticial pudiendo contribuir al deterioro de la función renal en estos enfermos.

Palabras clave: **Mastocitos. Glomerulonefritis mesangiocapilar tipo I. Morfometría.**

INTRODUCTION

Mesangiocapillary glomerulonephritis type I (MCGN-I) is a well-defined histopathologic entity that, although it may be found in a variety of clinical settings^{1,2}, is usually idiopathic³. This disease is characterised morphologically by the presence of granular, subendothelial electron-dense material presumed to be immune deposit⁴. Although morphometric investigations confirmed prominent tubulointerstitial fibrosis in this glomerulopathy⁵ the fibrogenic mechanisms operating locally in the kidney are not well understood at the present time⁶. A great number of cytokines and growth factors produced by the interstitial leukocyte subpopulations are probably involved in tubulointerstitial fibrosis⁷. Recent evidence also suggest a role for mast cells in the pathogenesis of renal scarring in various glomerulopathies⁸⁻¹¹. Mast cells contain inflammatory mediators such as histamine, prostaglandin, leukotriens, plate-

let activating factors and basic fibroblast growth factor¹⁰⁻¹². Although mast cells are well known to cause type I hypersensitivity¹³ and play a role in chronic inflammatory diseases such as scleroderma, rheumatoid arthritis and inflammatory bowel diseases⁸⁻¹⁴, the role of these cells in tubulointerstitial damage in glomerulonephritis is not fully understood¹¹. The involvement of mast cells in the process of renal interstitial fibrosis has not been investigated, because these cells are not easily detected by routine histochemical staining⁸. Recently, it is suggested that immunostaining with anti-tryptase monoclonal antibody detects almost all the mast cells in the renal interstitium^{10,15,16}.

Therefore, the present study was undertaken to ascertain whether mast cells have a role in tubulointerstitial fibrosis in MCGN-I and to examine the relationship between mast cells and interstitial α -smooth muscle actin (α -SMA) expression as well as interstitial infiltrates.

PATIENTS AND METHODS

Patients

Fourteen renal biopsy specimens from patients with MCGN-I were examined by percutaneous renal biopsy. Most of our patients were young adults: the mean age was 36.8 ± 8.4 and the male to female ratio was 6:8. The mean duration of the disease prior to biopsy was 6.5 month. Morphological diagnosis of MCGN-I was established independently by two experienced nephropathologists and based on light microscopy, immunofluorescence and electron microscopy. As a control 10 biopsy specimens of the kidneys removed because of trauma were used (the male to female ratio was 7:3, the mean age was 38.1 ± 7.2). None of the persons from whom renal tissue originated were known to have had previous or actual renal disease. Before the quantitative examination was carried out, all control specimens were histologically examined by a nephropathologist and found to be normal renal tissue.

Light microscopy

Tissue specimens were embedded in paraffin, sections cut precisely at 4 μ m, and stained by hematoxylin and eosin, periodic acid-Shiff (PAS)-alcian blue, trichrome light green (Masson), and by silver impregnation (Jones). Thickness of each section was controlled according to the method described by Weibel¹⁷.

Immunofluorescence microscopy

Tissue was snap frozen, sectioned at 5 μ m and fixed in 95% alcohol for 10 min. Sections incubated with FITC-antisera (Hoechst) to human IgG, IgA, IgM and complement (C3) were viewed on Carl Zeiss (Jena) NU-2 microscope, using and HBO 200 lamp and proper filters.

Electron microscopy

Tissue was fixex in glutaraldehyde, post-fixed in 1% osmium tetroxide, embedded in epon and sectioned on a LKB ultratome. Sections were stained by lead citrate and uranyl acetate, and viewed in a JEM 100B electron microscope.

Immunohistochemistry

The preparation and staining of tissue sections for immunohistochemistry (an indirect StreptAB-Complex/HRP technique) was carried out as follows: paraffin-embedded tissues, after deparaffinization and rehydration were reacted for 5 minutes with 3% hydrogen peroxide in distilled water. If necessary, pre-treatment with Trypsin (DAKO) for 15 minutes at 37 °C was performed (mouse monoclonal antihuman mast cell tryptase antibody and monoclonal antibody anti-CD68). After blocking by normal rabbit serum (DAKO) during 20 minutes (dilution 1:5), the sections were incubated with the following solutions, each followed by TBS washing:

- appropriately diluted mouse antihuman monoclonal antibodies (in a moist chamber for 30 minutes at room temperature),
- biotinylated rabbit anti-mouse immunoglobulin (DAKO) diluted 1:600 in TBS for 20 minutes, and
- StreptABComplex/HRP (DAKO) for 30 minutes prepared according to the instruction. The final reaction was achieved by incubating the sections with 3.3'-diaminobenzidine (DAB tablets DAKO) 0.5 mg/ml Tris-HCL buffer, pH 7.6, containing 0.02% hydrogen peroxide, for 10 minutes. After washing, sections were counter-stained with hematoxylin and coverslipped.

The monoclonal antibodies (DAKO) employed, their specific reactivities and dilutions are listed in table I.

Table I. Monoclonal antibodies employed and their specificity

Monoclonal antibody	Specificity	Source	Dilution
Anti-tryptase (AA-1)	Mast cells	Dako	1: 100
α -SMA	Myofibroblasts, smooth muscles	Dako	1: 50
CD 45RB	All leucocytes	Dako	1: 100
CD 43+	Pan - T cells	Dako	1: 100
CD 20+	Pan - B cells	Dako	1: 100
CD 68+	Monocytes/macrophages	Dako	1: 100

Tissue control for immunostaining

For each MoAb and for each sample a positive control was processed (for mast cells paraffin-embedded sections of nasal polyp where mast cells were found with toluidine blue staining, paraffin-embedded sections of surgically removed lymph node for interstitial leukocyte infiltrates and vascular smooth muscle cells of the renal biopsy specimens for myofibroblasts). Moreover, the following negative controls were used: 1) omission of primary antibody, 2) incubation with appropriately diluted mouse IgG (DAKO) as a first layer. The samples were prepared by the same method as described above. Specificity of labelling was shown by lack of staining in these samples.

Morphometry

Histological morphometry was performed by means of image analysis system consisting of a IBM-compatible computer equipped with an optical mouse, Indeo Fast card (frame grabber, true-colour, real-time), produced by Indeo (Taiwan), and colour TV camera Panasonic (Japan) linked to a Carl Zeiss Jenaval microscope (Germany). This system was programmed (program MultiScan 8.08, produced by Computer Scanning Systems, Poland) to calculate:

- the number of objects (semiautomatic function)
- the surface area of a structure using stereological net (with regulated number of points).

The immunophenotype of mast cells and leukocyte interstitial infiltration was determined by counting all positive cells for each monoclonal antibody (semiautomatic function) in a sequence of ten consecutive computer images of 400 x high power fields - 0.0047 mm² each. Mast cells were scored positive when displayed cytoplasmic granules stained positively with anti-tryptase monoclonal antibody. The only adjustments of field were made to avoid glomeruli and large vessels. The results were expressed as a mean number of immunopositive cells per mm².

Interstitial myofibroblasts were identified by their morphology and positive staining with anti- α -SMA. The interstitial immunoperoxidase staining for α -SMA was measured using point counting method, which is an adaptation of the principles of Weibel¹⁷. The point spacing being 16 μ m. Total number of the points of a net was 169, and total area was 36,864 sq. μ m. Under the net described above, 8-10 randomly selected adjacent fields of the renal cortex were investigated. Glomeruli and large blood vessels were neglected. The percentage of α -SMA positive

staining was an expression of the number of points overlying α -SMA positive areas as a percentage of the total points counted.

The same method was used to estimate interstitial volume in sections stained with Masson trichrome. The percentage interstitial volume was an expression of the number of points overlying renal cortical interstitium as a percentage of the total points counted.

Statistical methods

Differences between groups were tested using unpaired Student's t-test preceded by evaluation of normality and Levene's test. The Mann-Whitney U test was used where appropriate. Correlation coefficients were calculated using Spearman's method. Results were considered statistically significant if $p < 0.05$.

RESULTS

At the time of renal biopsy, a high percentage of patients with MCGN-I showed nephrotic syndrome or heavy proteinuria. Clinical renal impairment (serum creatinine greater than 1.5 mg/100 ml) was noted in 5 patients. Elevated blood pressure was observed in 9 MCGN-I cases. Haematuria accompanied proteinuria in 11 patients.

The morphometric data of the interstitial tryptase positive cells (figs. 1 y 2), interstitial expression of α -SMA, interstitial fibrosis and interstitial infiltrates appear from table II. The mean values of the interstitial tryptase positive cells, expression of α -SMA, interstitial volume, CD 68+, CD 45RB+, CD 43+ and

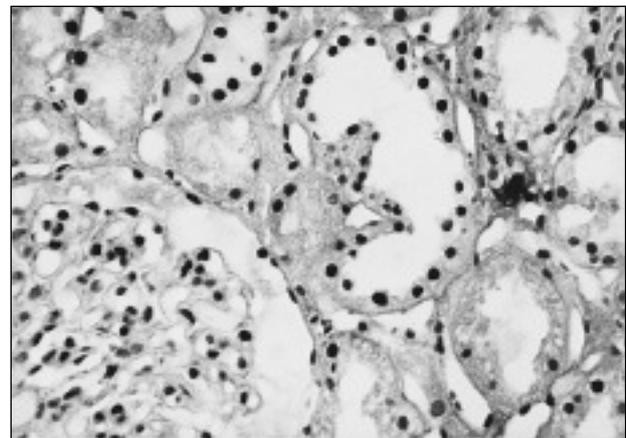


Fig. 1.—Immunostaining with anti-tryptase monoclonal antibody of a control case. Mast cell between tubules in periglomerular area (x 400).

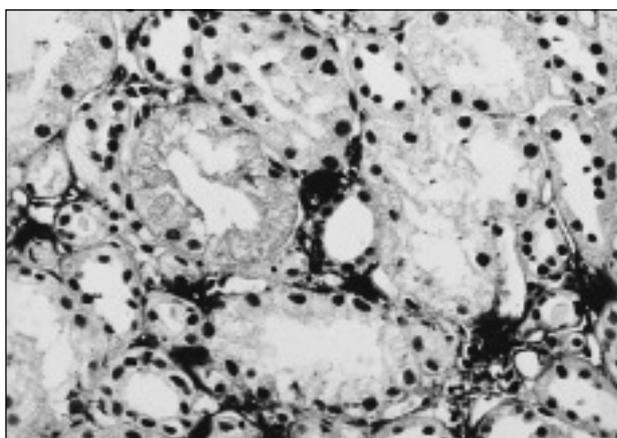


Fig. 2.—Immunostaining with ant-tryptase monoclonal antibody of in a patient with MCGN-I. Numerous polygonal in shape mast cells can be seen (x 400).

CD 20+ cells were in MCGN-I patients significantly increased in comparison with control group.

The correlations between interstitial tryptase positive cells and interstitial expression of α -SMA, interstitial volume, serum creatinine, proteinuria as well

as interstitial infiltrates are shown in table III. In MCGN-I group there were significant positive correlations between interstitial tryptase positive cells and interstitial expression of α -SMA, interstitial volume, serum creatinine as well as CD 43+ and CD 68+ cells (respectively: $r = 0.77, p < 0.002, r = 0.71, p < 0.005, r = 0.69, p < 0.01, r = 0.6, p < 0.03, r = 0.67, p < 0.01$). The correlations between interstitial tryptase positive cells and CD 45+, as well as CD 20+ cells were positive, but they have not reached statistical significance. No significant correlation was noted between interstitial mast cells and proteinuria. Similarly, correlations between all these parameters in controls were weak and not significant.

DISCUSSION

It was well documented that irrespective of the nature of primary disease the decline in renal function correlates better with the degree of tubulointerstitial injury (as measured by the relative interstitial volume) than with the glomerular changes¹⁸⁻²¹. The idea that mast cells are important to the pathogenesis of renal interstitial fibrosis was recently confirmed in

Table II. Morphometric data of interstitial mast cells, expression of α -SMA and a interstitial infiltrates in MCGN-I group and controls

Number of cases	α -SMA (%)	Interstitial volume (%)	Number of immunopositive cells per 1 mm ²				
			Tryptase+	CD 68+	CD 45RB+	CD 43+	CD 20+
Controls [n = 10]	0.52 \pm 0.26	10.08 \pm 1.25	1.24 \pm 0.86 (0.76%)	33.96 \pm 17.91 (20.92%)	83.43 \pm 20.91 (51.41%)	42.35 \pm 17.05 (26.1%)	1.31 \pm 1.52 (0.81%)
MCGN-I [n = 14]	16.38 \pm 12.37	23.22 \pm 9.67	19.65 \pm 12.2 (2.88%)	98.81 \pm 39.28 (14.47%)	311.57 \pm 126.29 (45.64%)	232.93 \pm 135.1 (34.12%)	19.75 \pm 8.81 (2.89%)
P value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Data are expressed as mean \pm standard deviation.
Data in parentheses are percentages of total immunopositive cells.

Table III. The correlations between tryptase positive cells and interstitial expression of α -SMA, interstitial volume as well as interstitial infiltrates in MCGN-I and controls

Correlation between	MCGN-I	Controls
Interstitial tryptase positive cells and interstitial expression of α -SMA	$r = 0.77, p < 0.002$	$r = 0.77, p = 0.8$ (NS)
Interstitial tryptase positive cells and interstitial volume	$r = 0.71, p < 0.005$	$r = 0.25, p = 0.4$ (NS)
Interstitial tryptase positive cells and CD 68+ cells	$r = 0.67, p < 0.01$	$r = 0.11, p = 0.7$ (NS)
Interstitial tryptase positive cells and CD 45RB+ cells	$r = 0.45, p = 0.1$ (NS)	$r = 0.10, p = 0.7$ (NS)
Interstitial tryptase positive cells and CD 43+ cells	$r = 0.6, p < 0.03$	$r = 0.07, p = 0.8$ (NS)
Interstitial tryptase positive cells and CD 20+ cells	$r = 0.01, p = 0.69$ (NS)	$r = 0.44, p = 0.2$ (NS)
Interstitial tryptase positive cells and serum creatinine	$r = 0.69, p < 0.01$	$r = 0.22, p = 0.4$ (NS)
interstitial tryptase positive cells and proteinuria	$r = 0.06, p = 0.65$ (NS)	

IgA-nephropathy¹⁰, in membranous nephropathy, diabetic nephropathy, focal segmental glomerular sclerosis and lupus nephritis⁸. To our knowledge, however, no data have documented quantitatively interstitial staining for mast cells in representative group of MCGN-I.

Our morphometric study showed that average number of interstitial tryptase positive mast cells was in MCGN-I patients significantly increased as compared with control group. Similar results were obtained by Hiromura y cols.⁸, in membranoproliferative glomerulonephritis, however, in this study only 5 patients were investigated. Moreover, no information was included if these cases were by electron microscopy identified as MCGN-I subgroup.

We observed tryptase positive cells in the interstitium between tubules, around glomeruli and blood vessels. No mast cells were found in the medullary interstitium and in glomeruli. Similar distribution of mast cells was reported in IgA nephropathy¹⁰. Contrary to our results in proliferative glomerulopathies some mast cells were detected in the medullary interstitium⁸. Similarly like in IgA nephropathy¹⁰ in the present study the average number of mast cells was lower than the average number of T lymphocytes, but in contrast to these authors, the mean number of mast cells was relatively small in comparison with the number of interstitial monocytes/macrophages. It must be noted, however, that in cited study the difference between these cells was not significant.

Whereas our results showed that mast cells were one of the constitutive interstitial cell types in MCGN-I, a major finding in this study was the demonstration that there were significant positive correlations between the number of interstitial mast cells and interstitial volume as well as between interstitial mast cells and serum creatinine concentration.

Although these findings are consistent with earlier suggestions that mast cells might play a role in the development of interstitial fibrosis and, in the process, may contribute to the renal deterioration^{8,9}, we agree with point of view of Ehara and Shigematsu¹⁰ that at the present time is not possible decide whether or not mast cell increased as the result of fibrosis. The precise mechanisms of renal interstitial fibrosis and the role of mast cells in this process are not clear¹¹. Mast cells have been shown to produce cytokines and growth factors that may contribute to fibrosis^{22,23}, whereas heparin and tryptase may enhance fibroblasts migration and proliferation^{24,25}. Moreover, fibroblasts and mast cells are known to influence each other^{12,26} and it is suggested that mast cell granule synthesis is also dependent on fibroblasts²⁷. Fibroblasts secrete mast cells growth factors,

for example c-kit ligand²⁶. Recently, Ehara and Shigematsu showed using double-labelled immunohistochemistry that some tryptase-positive mast cells had basic fibroblast growth factor in their cytoplasm¹⁰.

On the other hand, we found positive correlations between mast cells and interstitial T lymphocytes as well as monocytes/macrophages. Similar results were obtained by Toth y cols., in rapidly progressive glomerulonephritis¹¹. Also in study of Hiromura y cols., interstitial mast cells correlated well with the degree of tubulointerstitial leukocyte infiltration⁸. The role of leukocyte infiltrates in tubulointerstitial damage in human MCGN-I is well documented²⁸, but the relationship between interstitial mast cells and leukocytes is less clear²⁷. In skin with scleroderma mast cell proliferation is considered to involve IL-3 and IL-4 stimulation by T lymphocytes²⁹. It is noteworthy, that only tryptase positive mast cells have been suspected to be dependent on the T lymphocyte³⁰. Furthermore, we observed significant positive correlation between the mast cell count and interstitial expression of α -SMA. This result appears to be consistent with data of Toth y cols., who revealed similar relationship in rapidly progressive glomerulonephritis¹¹. Although it has been confirmed that interstitial α -SMA positive cells play a role in the development of interstitial fibrosis in MCGN-I, as they may act as active, extracellular matrix producing cells^{31,32}, the relationship between mast cells and interstitial myofibroblasts remains to be shown.

In conclusion, our findings demonstrate that mast cells are one of the constitutive cell types in the interstitium of MCGN-I. Additionally, significant positive correlations between interstitial mast cell count and relative interstitial volume as well as serum creatinine concentration suggest the role of these cells in the development of interstitial fibrosis and, in the process, may contribute to the renal deterioration in patients with MCGN-I.

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